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14. ABSTRACT
Task 1 : Evaluate optimal short term storage parameters for stimulated and unstimulated leukapheresis (donor lymphocytes) and bone marrow products, including the type of storage media and the cell concentration, in addition to temperature and duration of storage before processing or infusion.

Task 2: The NMDP has developed an algorithm that "predicts' high resolution HLA typing results on donor samples that exist in the Registry with only low or intermediate results reported. Perform validation of the NMDP algorithm by selecting donors randomly from our Registry that have low or intermediate DRB1 typing results and using the algorithm to predict the high resolution results and test the ability of the algorithm to predict KIR ligand mismatching in the absence of existing HLA-C locus results.

15. SUBJECT TERMS
Research in HLA Typing, Hematopoietic Stem Cell Transplantation and Clinical Studies to Improve Outcomes

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FINAL REPORT
PERFORMANCE / TECHNICAL REPORT

Office of Naval Research

And

The National Marrow Donor Program
3001 Broadway Street N.E.
Minneapolis, MN 55413
1-800-526-7809

20071101394

Task 1: Product Validation

Description:

The objective of this study is to evaluate optimal short term storage parameters for stimulated and unstimulated leukapheresis (donor lymphocytes) and bone marrow products, including the type of storage media and the cell concentration, in addition to temperature and duration of storage before processing or infusion.

Project 1. Effects of Media Storage and Cell Concentration

Stimulated and unstimulated leukapheresis and bone marrow products that are representative of products collected and provided for NMDP transplant patients will be purchased. Aliquots of the product will be stored in different storage media at varying cell concentrations per mL of media. Standard graft characterization parameters will be tested.

Objectives:

1. Transportation factors: Determine the effects of different types of tissue media, nucleated cell concentration on CD34+ cell, CD3+ and total nucleated cell viability, and CFU-GM frequency during transport from collection sites to the transplant centers.

2. Overnight storage factors: Determine the effects of different type of tissue media, type of storage bags (gas permeable or non gas permeable), nucleated cell concentration on CD34+, CD3+ cell and total nucleated cell viability, and CFU-GM frequency during overnight storage.

Project 2. Effects of Time and Temperature

Stimulated and unstimulated leukapheresis and bone marrow products that are representative of products collected and provided for NMDP transplant patients will be purchased. Aliquots of the product will be stored at varying lengths of time and temperature. Standard graft characterization parameters will be tested.

Objectives:

1. Temperature factors: Determine the optimal short term storage temperature to preserve nucleated cell count, percent viable TNC, CD34+ and CD3+ cells, CFU-GM frequency and sterility.

2. Time factors: Determine the effect of time on nucleated cell count, percent viable TNC, CD34+ and CD3+ cells, CFU-GM frequency and sterility.

Summary of Accomplishments:

Executive Summary:

Hematopoietic stem cell (HSC) transplants from unrelated donors are routinely used to treat patients with hematologic malignancies. HSC products are usually collected off site and require transport to the transplantation centers. The impact of transport conditions on HSC graft

composition during short-term storage has not been well described. This study evaluated the short-term handling and storage conditions for marrow, G-CSF mobilized peripheral blood stem cell (PBSC) and peripheral blood mononuclear cell (PBMC) products. The study examined five bone marrow (BM), four PBSC and five PBMC products from healthy volunteers. Products were stored at 4°C or 20°C (RT) for up to 72 hours Product samples were evaluated at 0,24,48 and 72 hours for white blood cells (WBC), viability, proliferative potential (CFC assay), CD34+ cells, monocytes (CD14+) and lymphocyte subsets (CD3+, 4+, 8+, 52+, 56+, 20+) during storage. Descriptive statistics and multivariate repeated measures analysis were performed for each of the variables and products. Percent cell viability, CD34+/µl and stem cell content CFU-GM/10^6 at t=0 through 72 hours are summarized below.

Total 7AAD Viability % *					
		t=0	t=24	t=48	t=72
PBSC	4°C	94.2	91.9	85.8	72.1
	20°C	94.2	77.1	46.6	39.3
BM	4°C	97.9	94.3	99.4	96.5
	20°C	97.9	91.8	93.1	93.8
CD34+/µl % *					
		t=0	t=24	t=48	t=72
PBSC	4°C	100.0	99.8	98.1	83.2
	20°C	100.0	71.4	19.0	4.6
BM	4°C	100.0	110.6	74.1	77.6
	20°C	100.0	154.2	94.3	105.6
CFU-GM/10^6 % *					
		t=0	t=24	t=48	t=72
PBSC	4°C	100.0	152.7	162.4	38.2
	20°C	100.0	61.8	9.7	0.0
BM	4°C	100.0	87.0	32.6	26.1
	20°C	100.0	43.5	32.6	30.4
* Base line percentage at t=0 hrs					

Cell viability, stem cell, monocytes and lymphocyte (except for CD8+ and CD20+) content for PBSC products decreased during 72 hours regardless of storage temperature (p=0.0001). The rate of degradation was worse in products stored at RT (p ≤0.001). For BM products; WBC, cell viability, CFU-GM, and CD4+ cell content decreased over 72 hours with more significant decreases in WBC and CFU for products stored at RT. Unlike PBSC, BM products stored at RT did not have significantly lower HSC content or viability than BM products stored at 4°C. The number of viable CD34+/ul was not affected by storage condition over time for BM products, but the quality of HSC as measured by CFU significantly deteriorated after 72 hours (p=0.0001). PBMC cell viability decreased over 72 hours with more significant decreases in products stored at RT. The HSC quality as measured by changes in product white blood cell count, viable CD34+ cells, cell viability, CFU colonies, monocytes and lymphocyte subsets during storage and between temperatures worsened over time regardless of storage condition. The primary objective of the study was to generate data to precisely define optimal and acceptable methods for handling of hematopoietic stem cells prior to infusion. Therefore, refrigerated storage of PBSC and BM products appears to optimize the quality and quantity of the product. This is particularly relevant for PBSC products that may require multiple days for collection and/or transport.

Introduction

Allogeneic hematopoietic stem cell transplants from unrelated donors are routinely used to treat patients with hematologic malignancies. These stem cell products, bone marrow (BM) and peripheral blood stem cells (PBSC) are generally collected off-site and require transport from the collection sites to transplantation centers. Currently, PBSC is the most requested product for unrelated donor transplantation through the National Marrow Donor Program (NMDP). Many of these PBSC collections are two-day procedures resulting in overnight storage of the first product at the apheresis center. It is also common, particularly if products arrive late in the business day, for either PBSC or marrow products to be held overnight at the receiving transplant center. Currently, the NMDP stipulates only that marrow should be infused within 24 hours of collection to maintain optimal product integrity, but transplant center practices vary.

Any of several factors including cell concentration, cellular composition, additives, temperature and duration of storage potentially affect stem cell viability *in vitro*. To date, however, relatively few publications addressing appropriate means of maintaining stem cell viability or optimal storage environment conditions exist.

This prospective study evaluated the short-term handling and storage conditions for stimulated and unstimulated leukapheresis (donor lymphocytes) and bone marrow products, including the type of storage media and the cell concentration, in addition to temperature and duration of storage before processing or infusion.

This study represents a collaborative effort between two organizations: The NMDP and the International Society for Cellular Therapy (ISCT). The NMDP/ISCT collaboration was developed to gather scientific data in support of guidelines to be proposed for optimal and acceptable methods for handling human marrow and PBSC prior to infusion or cryopreservation. Prior to defining the study design a survey was conducted to determine the current "state of the art" processing of hematopoietic stem cell (HSC) products. Therefore, a panel of experts including Donna Przepiorka, M.D., Linda L. Kelley, Ph.D., and Scott Burger, M.D. developed the survey (Appendix 1), distributed it to ISCT constituents and received over 100 responses. The survey results were summarized (Appendix 2), reviewed by the NMDP/ISCT design team and incorporated into the scope of work for analysis. The scope of work formed the basis for the request for proposal entitled: "Validation of Short-Term Handling and Storage Conditions for Marrow and Peripheral Blood Stem Cell Products".

Methods:

The contract for "Validation of Short-Term Handling and Storage Conditions for Marrow and Peripheral Blood Stem Cell Products" was awarded to the laboratory of Dr. Grace Kao, Assistant Medical Director, Connell& O'Reilly Families Cell Manipulation Core Facility (CMCF), Dana-Farber Cancer Institute (DFCI). Between October 1, 2006 and March 19, 2007 the CMCF at DFCI successfully recruited donors for a total of thirteen PBMC, PBSC, and BM products for

this study. This included a total of four PBMC products, four G-CSF mobilized PBSC/stem cell products and five bone marrow products from four healthy donors were evaluated in this study.

Product Collection

For collection of PBSC, PBMC, and BM, healthy volunteer donors were recruited from area medical institutions and apprised of the potential risks and benefits of participation in the study. IRB approved informed consent was obtained for collection of all products.

PBSC products were mobilized via self administered daily subcutaneous injections of filgrastim ($10\mu g/kg/day$) for a total of 5 days. PBSC and PBMC were collected using a COBE Spectra according to established procedures. Three to four total blood volumes (12-20 liters) were processed for each collection.

BM aspirates were obtained by a BMT service physician with a 12-gauge-aspiration needle inserted into the posterior superior iliac crest. Fifteen to twenty milliliters of BM aspirate was collected in 60cc syringe diluted with approximately 3.5ml of Lactated Ringers solution and 300IU of heparin.

Product Evaluation

Samples of each product type; marrow, PBSC and PBMC, included in the study adhered to the matrix of composition/storage conditions shown in Table 1 A and B. Briefly, cell concentration was maintained as described for the study. Each PBMC and PBSC product was divided and stored in three different conditions (Plasmalyte+Heparin [PH], Plasmalyte+ACD [PA], and Plasmalyte+Heparin+ACD [PHA]). Due to product volume and the survey results, bone marrow products were only stored in PH condition. Depending on the volume of apheresed products and BM, approximately 12 to 20 milliliters of the product were aliquoted and designated to one of the three storage compositions and temperatures as described in Table 1. Product samples at each composition/storage condition were taken for tests at each of the time points. In order to simulate actual product likely to be encountered in a transplant setting, the laboratory prepared BM and PBSC product samples with cellular concentrations ranges: Marrow: 13.7×10^6 WBC/mL - 26.3×10^6 WBC/mL, PBSC: 126×10^6 WBC/mL - 388×10^6 WBC/mL and PBMC: 10.0×10^6 WBC/mL - 50.0×10^6 WBC/mL.

Required sample assessments performed at each time point for all samples are summarized in Table 2. Briefly, physical characteristics were assayed by routine hematopoietic stem cell processing tests for pH, lactate level, osmolality, free hemoglobin, and potassium level. Proliferative potential was evaluated via CFU assay, CD34+ enumeration and viability (7AAD and trypan blue). CFU data were analyzed as the sum of all subtypes. Subgroup analysis for CFU-GM and CFU-GEMM was also analyzed individually. Immunophenotyping was conducted using flow cytometry to identify the cellular subset composition of the products, i.e. CD3+, CD4+, CD8+, CD14+, CD52+, CD56+, and CD20+. Sterility measurements were assayed at 72 hours for aerobic and anaerobic bacteria and fungi/mold. Finally, manual cell differential and hematocrit were also performed. The concentration of neutrophils, lymphocytes,

and monocytes were estimated based on the total WBC multiplied by the percentage of cell types.

Matrix of Composition/Storage Conditions for PBSC and PBMC				
Plasmalyte	Heparin	ACD-A	Storage Temperature	Time (Hour)
10%	10 IU/mL	1:10	4° C	0 24 48 72
10%	10 IU/mL	1:10	20° C	0 24 48 72
10%	10 IU/mL		4° C	0 24 48 72
10%	10 IU/mL		20° C	0 24 48 72
10%		1:10	4° C	0 24 48 72
10%		1:10	20° C	0 24 48 72

A.

Matrix of Composition/Storage Conditions for Marrow				
Plasmalyte	Heparin	ACD-A	Storage Temperature	Time (Hour)
10%	10 IU/mL		4° C	0 24 48 72
10%	10 IU/mL		20° C	0 24 48 72

B.

Table 1. Study design matrix

Required sample assessments were performed at each time point for all samples				
Physical Characteristics	Proliferative Potential	Immunophenotyping	Sterility Measurements (at T=72 hours only)	Cell Counts
Visual inspection (color, notation of any obvious clots or aggregates), pH, Lactate level, Osmolality, Free hemoglobin and Potassium level	CFU assay, CD34+ content (Methodology may be other than FLOW cytometry.), Viability (both trypan blue and 7-AAD), TNC and CD34+	Panel to include, at minimum, the following: CD34, AC133, CD3, CD4, CD8, CD52, CD25, CD56 and either CD19 or CD20.	Aerobic and anaerobic bacteria and Fungi/mold	Manual differential and hematocrit

Table 2.
Required sample assessments performed at each time point for PBSC, PBMC and BM products.

Statistical Analysis

Descriptive statistics were performed for each of the variables for each type of product (PBMC, PBSC, and BM). Multivariate linear regression analysis was also performed for the same variables for each type of product separately using PROC MIXED in SAS. Each regression model included two factors (Temp: 4°C, 20°C; Storage Composition: PHA, PH, PA), four time points (0, 24, 48, 72 hours) and 2-way interaction terms. Distributions of residuals as well as Y variables were investigated and an appropriate transformation (e.g., log_e, log_{10} or square root) was selected wherever needed. Multiple outcomes were not adjusted in the level of statistical significance and a two-sided p-value less than or equal to 0.05 was regarded as statistically significant.

Results

Cellular Viability and Stem Cell Content

Percent cell viability, CD34+/μl and stem cell content as measured by CFU-GM/10^6 at t=0 through t=72 hours are represented graphically in Figure 1 for PBSC and BM. Figure 1A graphically represents cellular viability for all three products. The overall analysis revealed decreases in cell viability, stem cell, monocyte and lymphocyte (except for CD8+ and CD20+) content for PBSC products during 72 hours storage regardless of storage temperature (p=0.0001). The rate of degradation was worse in products stored at 20°C (p $\leq$0.001). There were no differences between the three storage media for PBSC and PBMC. For BM products, WBC, cell viability, CFU-GM, and CD4+ cell content decreased over 72 hours with more significant decreases in WBC and CFU for products stored at 20°C. Unlike PBSC, BM products stored at 20°C did not have significantly lower stem cell content or viability than BM products stored at 4°C. Although the number of viable CD34+/ul was not affected by storage condition over time for BM products, the quality of stem cells as measured by CFU significantly deteriorated after 72 hours (p=0.0001). For unstimulated PBMC products, WBC decreased significantly after 48 hour storage in both 20°C (p=0.0001) and 4°C. Total cell viability significantly decreased over 72
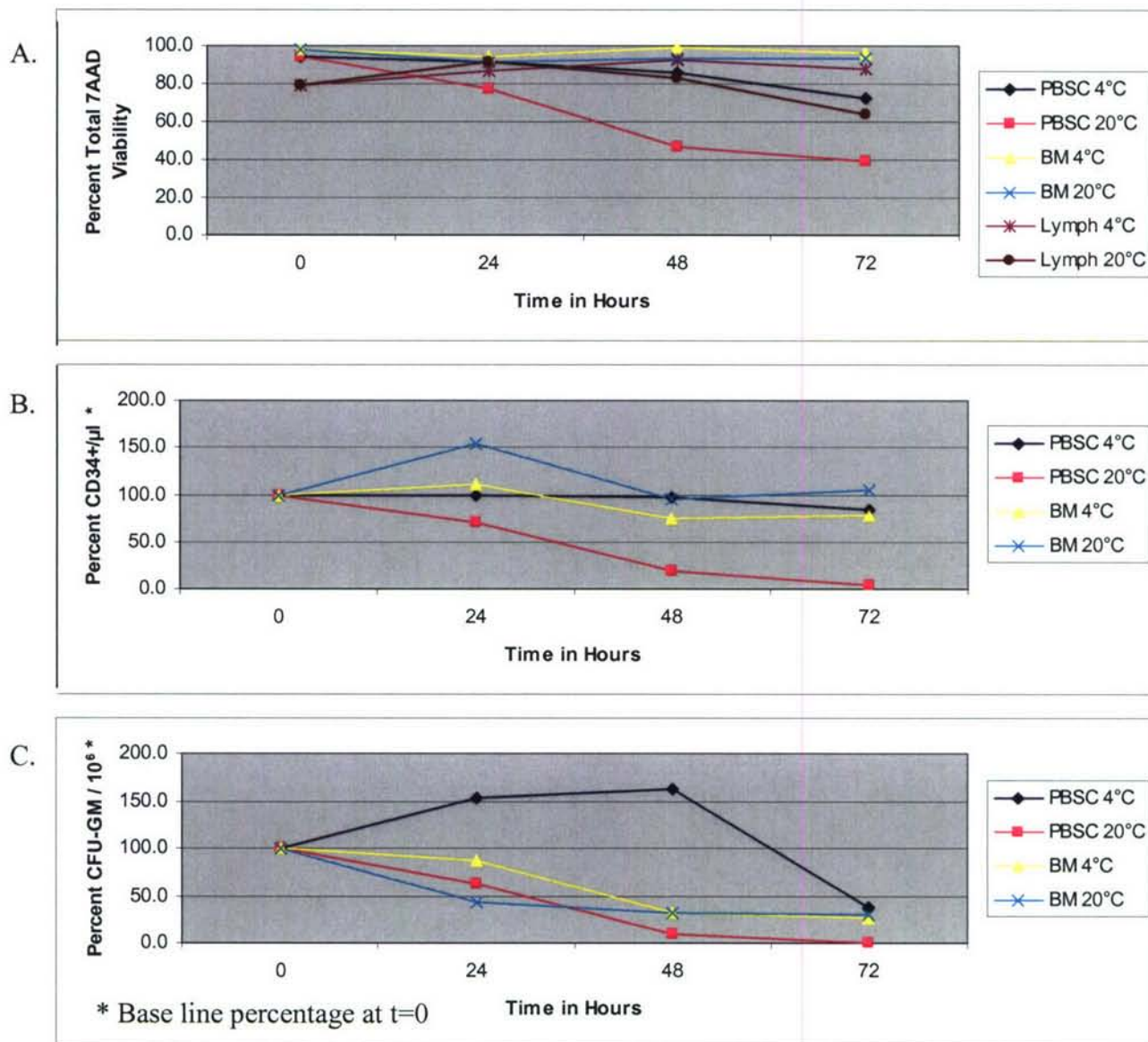
Figure 1.
Samples of each product type BM, PBSC and PBMC included in the study adhered to the following matrix of composition/storage conditions seen in Table 1. A. Percent total 7-AAD viability PBSC, PBMC and BM at time t= 0, 24, 48 and 72. B. Percent CD34+/μl PBSC and BM at time t= 0, 24, 48and 72. C. Percent CFU-GM/10^6 PBSC and BM at time t= 0, 24, 48and 72.

hours of storage time (p=0.004) and was significantly worse in products stored at 20°C (p=0.006). In addition, the stem cell quantity and quality, as measured by CD34+ and total CFU, both significantly decreased over time with 20°C products displaying significantly lower than 4°C products(p=0.004 and 0.02).

Hematocrit
For both PBSC and PBMC the hematocrit was noted to increase over time with products stored at 20°C whereas no notable change was observed with products stored at 4°C (p ≤0.0003). However for BM, platelet concentration and hematocrit were noted to increase at the same rate for products stored at both 20°C and 4°C (p=0.0016).

Physical Characteristics
Products stored with addition of ACD-A, such as PHA and PA, were more acidic (lower pH) than product stored without ACD-A, such as PH for PBSC and PBMC (p ≤0.002). In PBSC and PBMC the lactic acid and osmolarity of the products increased over 72 hours with a significantly higher increase in products stored in 20°C (p=0.0001). Potassium levels were increased at 72 hours and significantly more in 20°C stored product (p=0.0001), while free hemoglobin increased slightly over time but with no differences between the storage temperatures (p ≤0.003) for both PBSC and PBMC. There were no cell media content differences when the products were stored between the 3 described storage media over time. BM Products stored at 20°C were found to be more acidic; increasing over 72 hours (p=0.0001). The lactic acid and osmolarity increased over time with significantly greater increases observed in products stored in 20°C (p ≤0.005). Free hemoglobin and potassium level increased over 72 hours at an equal rate in both storage temperatures (p=0.0001).

Cellular Composition and Concentration
For both PBSC and PBMC flow cytometry studies found that cell content of CD3+, CD4+, CD52+ and CD56+ decreased over 72 hours (p ≤0.05). CD8+ was also decreased over 72 hours for PBMC, where CD14+ was decreased over 72 hours for PBSC (p ≤0.01). There was no change in CD20+ cell content over time for either PBSC or PBMC. Storage temperature did not affect the T cell, B cell, NK cell content. However, the decrease in CD3+, CD4+, CD8+, CD52+, CD56+, CD20+ cell concentration over time seen in PBSC was worse for product stored at 20°C when compared to 4°C storage (p ≤0.05). There were no differences observed between the three storage media conditions. For BM, flow cytometry studies only found changes over time in CD4+ concentrations(p= 0.03). The graft composition and concentration of all other cell types remained constant regardless of storage temperature or time.

Sterility Testing
14-day sterility tests were performed on all 72 hour-old products. None of the samples were culture positive.

Conclusion:

PBSC and PBMC products are affected more by storage temperature than BM products. The stem cell quality as measured by changes in product white blood cell count, viable CD34+ cells, cell viability, CFU colonies, monocytes and lymphocyte subsets during storage and between temperatures, worsened over time regardless of storage condition for all three products, however, at differing rates. The primary objective of the study was to generate data to precisely define optimal and acceptable methods for handling of hematopoietic stem cells prior to infusion. Therefore, short-term storage of PBSC, PBMC and BM products in refrigerated conditions appears to optimize the quality and quantity of the product. This is particularly relevant for the collection and storage of PBSC products that may require multiple days for collection and or transport. The results from this study have been submitted as an abstract to the 2008 BMT Tandem Meeting and will be written up for publication.

Task 2: Validation of the Expectation – Maximization (EM) Algorithm

Description:

The NMDP has developed an algorithm that "predicts' high resolution HLA typing results on donor samples that exist in the Registry with only low or intermediate results reported. A modified version of this algorithm predicts HLA results at loci where there are no typings based on existing typings at other loci and the ethnic-specific haplotype frequencies observed in the population.

It is our intention to incorporate this logic into the mechanisms used to select matched donors for patient searches. Incorporation of this logic would improve the specificity of donors that appear on patient's searches, which then decreases the costs and time necessary to identify the optimally matched donor. This logic will also be used to provide estimates of the likelihood of finding matched donors in the Registry including matching at loci where some donors in the Registry do not currently have typings.

A portion of the funding would be used to assist in the validation of the NMDP algorithm by selecting donors randomly from our Registry who have low or intermediate DRB1 typing results and using the algorithm to predict the high resolution results. The HLA typing results would be used to validate the accuracy of this method in an unbiased data set.

The remaining portion of the funding would be used to test the ability of the algorithm to predict KIR ligand mismatching in the absence of existing HLA-C locus results. Randomly selected donors from the Registry without HLA-C would be run through a modified version of the algorithm to predict the C locus KIR ligand status. The HLA intermediate resolution typing would validate the accuracy of this method in an unbiased data set.

A laboratory would perform the high resolution HLA-DRB1 testing and/or intermediate resolution HLA-B and C from stored samples of the donors. Quality control and performance criteria will be monitored by a Scientific Services Specialist. The results will be analyzed by a programmer in the Bioinformatics group to verify the accuracy of each prediction technique.

In addition to assisting with the validation of the algorithm, this typing project has potential to impact subsequent patient searches simply due to the increased level of resolution for the Registry donors whose typings have been upgraded. A portion of this typing may be selected on behalf of searching patients in order to further validate the approach and provide direct positive impact on these searches.

Summary of Accomplishments:

Executive Summary:

Prior to implementation of Haplogic, a large-scale validation HLA typing project was undertaken to calculate the accuracy of the Expectation Maximization algorithm predictions. An unbiased set of 2,500 minority donors the NMDP Registry who had intermediate resolution for DRB1 and

low/intermediate resolution for A and B were selected. The algorithm predicted the allele level results for HLA-A, B and DRB1. Then donor samples were prospectively high resolution typed for HLA-A, B, C, DRB1 and DRB3/4/5. The racial/ethnic composition of the study population was 31% African American (AFA), 26% Asian Pacific Islander (API), 39% Hispanic (HIS) and 4% Native American (NAM). Prediction quality was evaluated using the Receiver Operating Characteristic (ROC) method (1.0 is perfect prediction) with a cutoff at 75% likelihood of matched prediction. The overall ROC value was 0.7722. Values for the three largest minority groups were AFA=0.8033, API=0.7876 and HIS=0.732. The validation demonstrated that 4-digit DRB1 composition could be accurately estimated from lower level haplotype information for individuals in high HLA diversity minority groups.

Overall Summary:

Extensions to the Expectation Maximization (EM) algorithm were implemented that produced unbiased 2-digit HLA-A, B and 4-digit HLA-DRB1 haplotype frequency estimates from large sample populations (~5 million) having low/intermediate resolution A, B and DRB1 HLA typing. These haplotype frequency estimates were then used to predict the likelihood of allele level matching for donors and cord blood units appearing as potential (low resolution) matches on patient searches. These predictions comprised the most significant modifications to the NMDP Matching Algorithm leading to the launch of HapLogic.

Prior to implementation, a large-scale validation HLA typing project was undertaken to calculate the accuracy of the predictions. An unbiased set of 2,500 minority donors from the NMDP Registry who had intermediate resolution for DRB1 and low/intermediate resolution for A and B were selected. The algorithm predicted the allele level results for HLA-A, B and DRB1. Then the samples received prospective high resolution typing for HLA-A, B, C, DRB1 and DRB3/4/5.

The racial/ethnic composition of the donors was:

- 31% African American (AFA)
- 26% Asian/Pacific Islander (API)
- 39% Hispanic (HIS)
- 4% Native American (NAM))

The following laboratory activities were completed:

- Three laboratories were contracted to HLA type the 2500 samples for high resolution A,B,C,DRB1/3/4/5.
- Sample shipments occurred March through April 2005
- Typing results were completed in May 2005
- Laboratory performance was tightly monitored with blind quality control samples and turnaround time (TAT) was calculated.

a. QC error rate for all labs = 1.13% (less than 2.0% required)
b. TAT for all labs = 95.6% (greater than 90% required)

The modified EM algorithm used the A-B-DRB1 haplotype frequency estimates to predict the 4-digit DRB1 alleles based on the full A-B-DRB1 genotype. For each donor, a most-likely predicted 4-digit DRB1 allelic subtype was assigned a probability value ([0,1]). The results of the high-resolution typing were compared to the original (low/intermediate resolution) DRB1 type and the predicted DRB1 type, and the cohort was partitioned into 4 categories (1-discrepant, 2-matched prediction, 3-mismatched prediction, or 4-indeterminate/incomplete).

Prediction quality was evaluated by using the ROC method (Receiver Operating Characteristic: 1.0 is perfect prediction) with a cutoff at 75% likelihood of matched prediction. The overall ROC value was 0.7722. Values for the three largest minority groups were AFA=0.8033, API=0.7876 and HIS=0.732. The validation demonstrated that 4-digit DRB1 composition could be accurately estimated from lower level haplotype information for individuals in high HLA diversity minority groups.

The KIR ligand prediction analysis was not completed. Resources were shifted to support the extension of the haplotype estimation analysis to include allele level HLA-A, B, C and DQB1 prediction rather than only DRB1 as originally proposed.

State of the Art Survey

Greetings!

The National Marrow Donor Program® (NMDP) and the International Society for Cellular Therapy (ISCT) are collaborating on the design of a study intended to validate short-term handling and storage conditions for marrow and peripheral blood stem cell (PBSC) products.

The ultimate goal is to generate data to be used as the scientific basis for establishing optimal handling and storage condition guidelines for these products.

As a preliminary step, we are conducting this **brief** (4 question!) survey to assess several "state-of-the-art" cellular processing practices.

Your cooperation in providing us this feedback would be greatly appreciated. Please return your responses to Sue Flesch at the NMDP by either email **(sflesch@nmdp.org)** or **FAX at 612/362-3488** by **Monday April 3, 2006.**

Thank you in advance for your time and assistance.

_____ Domestic Laboratory
_____ International Laboratory

Name of Institution (optional) _____

1) What additive/diluent (include concentration/amount) does your laboratory routinely use for each of the following products?

Marrow

___ Plasmalyte @ _____
___ Normasol @ _____
___ Lactated Ringer's Solution @ _____
___ Normal Saline @ _____
___ Other (please specify): _____

PBSC

___ Plasmalyte @ _____
___ Normasol @ _____
___ Lactated Ringer's Solution @ _____
___ Normal Saline @ _____
___ Other (please specify): _____

State of the Art Survey

Unstimulated PBMC

____ Plasmalyte @ _____
____ Normasol @ _____
____ Lactated Ringer's Solution @ _____
____ Normal Saline @ _____
____ Other (please specify): _____

2) Which anticoagulants (and concentration) does your laboratory routinely use for each of the following products?

Marrow

____ Heparin @ _____
____ ACD-A @ _____
____ Combination of heparin and ACD-A @ _____ and @ _____
____ Other (please specify) _____ @ _____

PBSC

____ Heparin @ _____
____ ACD-A @ _____
____ Combination of heparin and ACD-A @ _____ and @ _____
____ Other (please specify) _____ @ _____

Unstimulated PBMC

____ Heparin @ _____
____ ACD-A @ _____
____ Combination of heparin and ACD-A @ _____ and @ _____
____ Other (please specify) _____ @ _____

3) What assay or methodology does your laboratory presently use to determine cell viability of the following?

TNC _____
CD34$^+$_____

State of the Art Survey

4) Which pre-infusion processing methods does your laboratory employ for each of the following products?

Marrow

____ Red cell depletion
____ Plasma depletion
____ CD34+
____ Other (please specifiy): _____

PBSC

____ Red cell depletion
____ Plasma depletion
____ CD34+
____ Other (please specifiy): _____

Unstimulated PBMC

____ Red cell depletion
____ Plasma depletion
____ CD34+
____ Other (please specifiy): _____

Summary of Survey Data

Cellular Processing "State-of-the Art" Survey Summary
NMDP/ISCT Product Validation Study
May-06

Domestic laboratories (45 total survey respondants)
International laboratories (31 total survey respondants)

1) What assay or methodology does your laboratory presently use to determine the viability of the following cells/subsets?

	Domestic laboratories	International laboratories	All laboratories
TNC			
Trypan blue	29 (64.44%)	11 (35.48%)	40 (52.63%)
7-AAD	8 (17.78%)	10 (32.26%)	18 (23.68%)
others mentioned:	acridine orange, ethidium bromide-acridine orange, Topro, propidium iodide colonly assays, FACS		
CD34*			
Trypan blue	9 (20.00%)	5 (16.13%)	14 (18.42%)
7-AAD	17 (37.78%)	13 (41.94%)	30 (39.47%)
others mentioned:	propidium iodide,Topro, CFU-GM, colony assay, FACS (BD or ISHAGE protocol or CD34 and CD45 positive cells)		

2) What additive/diluent does your laboratory routinely use for each of the following products?

	Marrow			PBSC			Unstimulated PBMC		
	Domestic laboratories	International laboratories	All laboratories	Domestic laboratories	International laboratories	All laboratories	Domestic laboratories	International laboratories	All laboratories
Plasmalyte	15 (33.33%)	3 (9.68%)	18 (23.68%)	17 (37.78%)	5 (16.13%)	22 (28.95%)	13 (28.89%)	1 (3.23%)	14 (18.42%)
Normasol	9 (20.00%)	0 (0.00%)	9 (11.84%)	7 (15.56%)	0 (0.00%)	7 (9.21%)	5 (11.11%)	0 (0.00%)	5 (6.58%)
Lactated Ringers	3 (6.67%)	0 (0.00%)	3 (3.95%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
Normal Saline	6 (13.33%)	7 (22.58%)	13 (17.11%)	4 (8.89%)	5 (16.13%)	9 (11.84%)	4 (8.89%)	6 (19.35%)	10 (13.16%)
Other	6 (13.33%)	9 (29.03%)	15 (19.74%)	6 (13.33%)	16 (51.61%)	22 (28.95%)	3 (6.67%)	11 (35.48%)	14 (18.42%)

3) Which anticoagulant does your laboratory routinely use for each of the following products?

	Marrow			PBSC			Unstimulated PBMC		
	Domestic laboratories	International laboratories	All laboratories	Domestic laboratories	International laboratories	All laboratories	Domestic laboratories	International laboratories	All laboratories
Heparin	32 (71.11%)	17 (54.84%)	49 (64.47%)	20 (44.44%)	7 (22.58%)	27 (35.53%)	11 (24.44%)	6 (19.35%)	17 (22.37%)
ACD-A	14 (31.11%)	10 (32.26%)	24 (31.58%)	38 (84.44%)	22 (70.97%)	60 (78.95%)	22 (48.89%)	17 (54.84%)	39 (51.32%)
Other	1 (2.22%)	2 (6.45%)	3 (3.95%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)

4) Which pre-infusion processing methods does yur laboratory employ for each of the following products?

	Marrow			PBSC			Unstimulated PBMC		
	Domestic laboratories	International laboratories	All laboratories	Domestic laboratories	International laboratories	All laboratories	Domestic laboratories	International laboratories	All laboratories
Red Cell Depletion	36 (80.00%)	22 (70.97%)	58 (76.32%)	19 (42.22%)	7 (22.58%)	26 (34.21%)	13 (28.89%)	2 (6.45%)	15 (19.74%)
Plasma Depletion	36 (80.00%)	17 (54.84%)	53 (69.74%)	36 (80.00%)	12 (38.71%)	48 (63.16%)	22 (48.89%)	5 (16.13%)	27 (35.53%)
CD34 Selection	6 (13.33%)	8 (25.81%)	14 (18.42%)	21 (46.67%)	20 (64.52%)	41 (53.95%)	4 (8.89%)	3 (9.68%)	7 (9.21%)
Other	8 (17.78%)	5 (16.13%)	13 (17.11%)	4 (8.89%)	7 (22.58%)	11 (14.47%)	3 (6.67%)	5 (16.13%)	8 (10.53%)